COLONIZATION OF DECIDUOUS LEAF LITTER BY BACTERIA AND TESTATE AMOEBAE

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I. INTRODUCTION

The aim of this paper is to provide qualitative and quantitative data on the colonization of aspen woodland leaf litter by bacteria and testate amoebae during the initial stages of decomposition. The study was carried out in the aspen woodland site described in PARKINSON and LOUSIER (1975) and colonization of both types of tree litter *(Populus tremuloides* Michx. and *P. balsamifera* L.*)* was studied.

PARKINSON and LOUSIER (1975) have given information on the amount of energy and nutrient material (in the form of plant litter) entering the aspen woodland soil and data are being collected on some of the types and biomass of the organisms present and their roles in utilizing the incoming energy and nutrients. Among the groups being studied are the aerobic heterotrophic bacteria and the testate amoebae (Protozoa, Rhizopoda, Testacea), the first group being an important group of primary decomposers and the second being an example of a range of organisms that actively prey on bacteria (STOUT, 1973).

There have been few ecological studies carried out on microorganisms colonizing leaf litter decomposing on the soil surface. MARTEN and POHLMAN (1942) looked at colonization of litter of six different tree species by bacteria and fungi in very controlled conditions and tried to relate it to chemical changes in the decomposing litter. SAITO (1966) dealt with the successive changes of the decomposition processes of beech leaf litter and the associated microbial succession. MINDERMAN and DANIELS (1967) analyzed quantitatively the colonization of oak litter by fungi, bacteria and actinomycetes. In an attempt to explain the interrelationships of litter microorganisms, REMACLE (1971) detailed the changes in the species composition of the litter bacteria and fungi during oak leaf decomposition under laboratory conditions. The bacterial research reported in this paper is comparable to the extensive work done at Meathop Wood and published in Hissett and GRAY (1973) and Gray, Hissett and DUXBURY (1974). There is scant information available concerning colonization of leaf litter by Testacea and concerning Testacea-bacteria interrelationships.

II. METHODS

The litter used was confined in litter bags (mesh size 3 mm, bag size 20 cm x 20 cm) as described in PARKINSON and LOUSIER (1975).

1) Bacteria

a) Enumeration of bacteria : 3 litter bags of each species of tree litter were removed from the site each sampling period (0, 1, 5, 12 months after burial) and 2 replicate samples were prepared from each bag. The samples were processed immediately after removal from the field or were stored at 4°C for no longer than 18 hours. 0.5 gram sub-samples were blended in 50 cc of sterile distilled water at full speed in a Waring blender for 5 minutes at a temperature of 4°C. Distilled water serial dilutions were prepared and three 1 cc aliquots from each of the last three dilutions were transferred to petri plates. 20 cc of molten, cooled modified leaf extract agar were syringed into the plates. Modified leaf extract agar was prepared as per Bunt and Rovira (1955) except that a 1:10,000 dilution of leaf extract replaced the soil extract. Actidione and mycostatin were added after autoclaving (GOODFELLOW et al., 1967). The bacterial plates were incubated for 3 weeks at 25°C.

b) Isolation of Bacteria : From the dilution plates which had fewer than 50 colonies every colony was isolated into sporulation broth (ATCC Catalogue, 1970) until 125 organisms were isolated or in the case of freshly fallen litter all organisms were isolated since numbers were very low. The organisms were stored in broth at 4°C until they could be examined.

c) Characterization of the Isolates : Organisms were streaked onto sporulation agar and isolated colonies were transferred to sporulation broth or to peptone yeast extract broth (ENSIGN, 1970) and incubated at 25°C on a shaker. A wet mount was observed using phase contrast microscopy to determine arrangement of cells and for motility. A Gram stain was prepared from the broth in order to observe cell size, shape and Gram reaction of young cultures (24-48 hours) and, where applicable, of old cultures (1-2 weeks). The size, shape, consistency and pigmentation of the colony were determined, as well as the formation of a pellicle, sediment, or turbidity in broth. Other tests included the formation of endospores and acid fastness for Gram + or Gram variable rods suspected to be *Bacillus* or *Mycobacterium*, oxidase (STEEL, 1961), oxidation or fermentation of glucose (HUGH and LEIFSON, 1953), utilization of cellulose (SKERMAN, 1967) ; hydrolysis of gelatin and starch, utilization of citrate, cellobiose and urea, production of H_2S, utilization of carbohydrates and/or proteins in litmus milk, and production of a fluorescent pigment on PSAF agar using Bacto dehydrated culture media.

d) Isolate Description :

Bacillus spp. - non-pigmented colony ; endospores observed ;
- non-sporulating cells which had a cellular morphology similar to that of *Bacillus*, but which could not be induced to sporulate.

Corynebacterium spp. - originated from plant material ; colony morphology was cream to pale yellow. Gram + cells which may be pleomorphic but not branching. Palisade, V or W arrangement of cells often observed.

- as above, but colony may be pale red rather than yellow.

Arthrobacter spp. - presence of cystites or a change in cell morphology from rod (usually Gram negative) to coccoid cells (Gram +) due to the age of the culture or a change in the nutrients provided for growth (ENSIGN, 1970).

Nocardia spp. - Gram + branching rods which with age become coccoid. Colony pasty rather than smooth and glistening as in *Arthrobacter* spp.

Streptomyces spp. - Gram + or - filamentous hyphae, colony usually dry and powdery on upper surface and produced a brown, water soluble pigment.

Micrococcus spp. - spherical cells, Gram + and approximately .5 - .75 µm in diameter. Non-pigmented colony. Did not ferment glucose.

Sarcina spp. - Gram + cocci, .5 - 1 µm in diameter arranged in tetrads or cubical packets.

Mycobacterium spp. - Acid fast Gram + rods which may be branching.

Cytophaga spp. - Gram (-) flexible rods, .3 - .5 x 2 - 15 µm. Weakened strip of cellulose paper in phytone broth.

Sporocytophaga spp. - Gram (-) rods which form ellipsoidal microcysts which had a highly refractile cell wall.

Yellow pigmented Gram (-) rods - Characteristics were variable, however if glucose was utilized it was oxidized and litmus milk was often turned alkaline with or without peptonization or reduction.

Orange pigmented Gram (-) rods - similar to yellow pigmented rods as described above except that colony was orange or in a few cases red.

Pseudomonas spp. - oxidized glucose, produced fluorescein pigment on PSAFand were motile.

Non-pigmented Gram (-) rods - composed primarily of organisms from the families Achromobacteriaceae, Rhizobiaceae and Pseudomonadaceae since glucose was oxidized if utilized at all.

2) Testacea

At each sampling time (1, 5, 8, 10, 12, 18 months after burial of litter bags), 5 litter bags of each species of tree litter were removed and returned to the laboratory where 4 replicate 1 g (wet wt) subsamples were taken from each bag. Each subsample was fixed (Bouin-Hollande), stained (Xylidine ponceau), blended for 10 sec at 3000 rpm and filtered through a millipore filter apparatus (as used by COÛTEAUX, 1967). All fields on all filters were examined microscopically and the Testacea identified and counted.

III. RESULTS AND DISCUSSION

In terms of numbers, the bacteria colonizing both aspen and balsam litter are similar (Tables 1 and 2). There are significant differences between the numbers present on freshly fallen leaves and those present at 1 month for both types of leaves, and also from 1 month to 5 months. The high proportion of chromogenic bacteria, including both Gram (+) and Gram (-) isolates, is typical for litter (STOUT, 1971). Stout found many of the airborne flora to be present in the litter and also suggested that the yellow and orange pigmented rods were less sensitive to ultra-violet light. In soil, *Bacillus* spp. have been reported to be the dominant genus (Goodfellow, 1968). As seen in Tables 1 and 2, *Bacillus* is present only in low numbers in litter within the first year.

The organisms isolated were, on the whole, active in the utilization of one or more of the following compounds : glucose, casein, gelatin, starch, citrate and urea. This may indicate a zymogenous flora which is present in larger numbers on litter containing easily utilizable compounds. The yellow and the orange pigmented bacteria are not present in such proportions in the humus (unpublished data). The concentration of proteolytic, amylolytic, cellulolytic and nitrogen-fixing bacteria is often higher in litter than in soil (REMACLE, 1971). The basis for grouping litter bacteria as zymogenous and soil bacteria as autochthonous (STOUT, 1972), may, however, have biochemical limitations.

Colonization of the 2 types of litter by Testacea is similar in terms of species and numbers of species (Tables 3 and 4). However, the major differences were the failure of *Euglypha laevis* and *E. rotunda* (very ubiquitous species and only 45 µm in size) to colonize *Populus balsamifera* litter as readily as they did *P. tremuloides* litter, and the relatively rapid colonization of *P. balsamifera* litter by the spined *E. compressa* and non-spined *E. compressa* forma *glabra* (rare species and about 90 µm in size).

No colonization was recorded before the 5-month sample. A 1-month sample was taken but the winter freeze and the first permanent snow had occurred and no Testacea were observed in the litter. The 5-month sample was taken less than 1 week after the initiation of spring thaw. In the first 18 months of colonization the Family Euglyphidae dominated (usually ⩾50 % of the species) the colonizing Testacea. After 18 months 2 species of Testacea (*Euglypha scutigera* and *Centropyxis aerophila* var. *sphagnicola*) had been recorded in *P. tremuloides* litter but appeared not to have yet invaded *P. balsamifera* litter.

It appears that colonization by Testacea is relatively rapid (after 18 months all the litter species recorded in LOUSIER (1975) for the A_0L layer are present ; it appears in some as yet unpublished data that the residence time of leaf litter in the A_0L layer in the aspen woodland soil is 3-4 years) despite certain biological limitations of the group, e.g., mineral requirements for test construction. After 18 months, 67-69 % of the species recorded form their tests from absorbed inorganic minerals, while the rest require sediment materials for their tests. The latter types of tests seem to lag somewhat more than the former in terms of appearance in the litter and significant reproduction (Tables 3,4,5).

Table 5 indicates that colonization of aspen litter by Testacea after 18 months is more successful in terms of total numbers despite there being no significant differences in testacean numbers between aspen and balsam leaves up to and including 12 months.

Colonization of aspen litter by Testacea was more successful also in terms of significant reproduction (significance measured by determining total numbers produced over 18 months – this method is in LOUSIER (1974) *P. tremuloides* litter had 7 testacean species reproducing significantly (*E. laevis, E. rotunda, E. cuspidata, T. enchelys, T. lineare, D. oviformis, C. aerophila* all under 65 µm in thickness) while *P. balsamifera* had only 4 (*A. catinus, T. lineare, P. acropodia, C. aerophila* - all under 65µm in thickness).

From 5 months to 8 months, litter moisture had dropped from 62-20 % and numbers of Testacea per species were reduced by about 60 %, but the number of species had tripled (from 2 to 6). However, total numbers of Testacea remained the same. From 8 to 10 months, a doubling of the litter moisture (however, 33-38 % moisture was still low) produced a significant increase in numbers of species and significant reproduction. Bacterial diversity had peaked (Tables 1 and 2) in terms of the study period, but in June (7-8 months after litter fall) bacterial numbers decreased (unpublished data) because perhaps of the higher soil surface temperatures and lower moisture. When considering Testacea as bacterial predators, it seems likely that the quality of the food base may determine the succession of testacean species, whereas the quantity of food and moisture may influence reproduction of testacean species.

There were significant increases in bacterial biomass from 0 to 1 and 1 to 5 months intervals, and in testacean biomass from 0 to 5, 8 to 10, 10 to 12, and 12 to 18 months intervals (Table 6). In comparison with fungal biomass data, e.g., 808 mg/m^2 at 5 months decomposition (VISSER and PARKINSON, 1975) and 2,398 mg/m^2 at 12 months decomposition (VISSER, 1971), the bacterial and testacean figures are quite low. The bacterial biomass figures, based on plate counts using a single isolating medium, probably represent underestimates of bacterial populations in leaf litter. In wiew of this, the differences between fungal and bacterial biomass referred to above may well not accurately reflect the field situation. Testacean biomass data indicate that while there are standing crops of Testacea at 5 and 8 months, a substantial increase in biomass occurred only by 10 months. This time lag is a normal phase of the growth curves of predatory populations, allowing development of the diversity and quantity of the food base, the bacteria.

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Table 1. Isolates and proportions of bacteria per gram dry weight of *Populus tremuloides* litter

Isolates	Freshly Fallen Litter	1 month	5 months	12 months
Bacillus sp.	0.10	0	0	0
Corynebacterium sp.	0.25	0.24	0.08	0.07
Arthrobacter sp.	0.08	0.12	0.03	0.16
Nocardia sp.	0.02	0.01	0	0.01
Streptomyces sp.	0.02	0	0.02	0
Mycobacterium sp.	0.02	0.01	0	0
Micrococcus sp.	0.02	0.02	0	0
Sarcina sp.	0	0	0.02	0.02
Cytophaga/Sporocytophaga	0.02	0.02	0.03	0.01
Yellow-pigmented Gram (–) rods	0.17	0.26	0.46	0.38
Orange-pigmented Gram (–) rods	0.10	0.19	0.11	0
Non-pigmented Gram (–) rods	0.22	0.13	0.25	0.30
Pseudomonas sp.	0	0	0	0.03
Total Numbers (x 10^6)	0.0052	1.26*	1737.6*	7305
% Moisture	20	24.1	47.8	60.2

* Indicates a significant difference (P = 0.05) from the value recorded at previous sampling time.

Table 2. Isolates and proportions of bacteria per gram dry weight of *Populus balsamifera* litter.

Isolates	Freshly Fallen Litter	1 month	5 months	12 months
Bacillus sp.	0	0.07	0	0
Corynebacterium sp.	0.15	0.02	0	0.05
Arthrobacter sp.	0.15	0.05	0.07	0.08
Streptomyces sp.	0	0.06	0	0.02
Micrococcus sp.	0.10	0	0	0.02
Cytophaga/Sporocytophaga	0.10	0	0	0.02
Yellow-pigmented Gram (–) rods	0.05	0.13	0.57	0.61
Orange-pigmented Gram (–) rods	0.45	0.34	0.12	0.12
Non-pigmented Gram (–) rods	0	0.33	0.23	0.11
Pseudomonas sp.	0	0.005	0	0
Unidentified sp.	0	0.005	0.02	0
Total Numbers (x 10^6)	0.027	1.18*	2291.5*	7280.0
% Moisture	20.0	25.7	46.8	61.1

* Indicates a significant difference (P = 0.05) from the values recorded at previous sampling time.

Table 3. Succession of species and changes in proportions of active and encysted forms for the colonization by aspen poplar leaf litter by Testacea (the number of individuals of Testacea in a given sample is expressed as a proportion of the total number per gram dry weight of litter)

	Populus tremuloides							
	5 mos	8 mos	10 mos		12 mos		18 mos	
Species	Activ.	Activ.	Activ.	Cysts	Activ.	Cysts	Activ.	Cysts
Arcella catinus	0	0.17	0.09	0.17	0	0.14	0.02	0.17
Euglypha laevis	0	0.17	0.09	0.17	0.08	0.14	0.06	0
E. rotunda	0	0.17	0.18	0.34	0.08	0.29	0.14	0.34
E. scutigera	0	0	0	0	0	0.14	0.02	0
E. compressa	0	0	0	0	0.17	0.14	0.06	0
E. compressa f. *glabra*	0	0	0	0	0.08	0	0.02	0
E. cuspidata	0.50	0.17	0.09	0.17	0.08	0.14	0.06	0.17
Trinema enchelys	0	0	0.09	0.17	0.08	0	0.08	0.17
T. lineare	0	0	0.09	0	0.08	0	0.25	0.17
Difflugiella oviformis	0.50	0.17	0.18	0	0.08	0	0.06	0
Phryganella acropodia	0	0	0.09	0	0.08	0	0.02	0
Heleopera petricola	0	0	0	0	0	0	0.06	0
Centropyxis aerophila	0	0	0	0	0.08	0	0.08	0
C. aerophila var. *sphagnicola*	0	0	0	0	0	0	0.02	0
C. sylvatica	0	0.17	0.09	0	0.08	0	0.04	0
No. of Species	2	6	9		13		15	
No. of individuals/g dry wt	182	204	550	300	1550*	905*	4951*	428*
% H_2O (wet wt)	63.1	16.8	33.4		48.4		58.0	

* Indicates a significant difference (P = 0.05) from value recorded at previous sampling time.

Table 4. Succession of species and changes in proportions of active and encysted forms for the colonization of balsam poplar leaf litter by Testacea (the number of individuals of Testacea in a given sample is expressed as a proportion of the total number per gram dry weight of litter)

	Populus balsamifera							
	5 mos	8 mos	10 mos		12 mos		18 mos	
Species	Activ.	Activ.	Activ.	Cysts	Activ.	Cysts	Activ.	Cysts
Arcella catinus	0	0.17	0.10	0.25	0.09	0.50	0.10	0.50
Euglypha laevis	0	0	0	0	0	0	0.05	0
E. rotunda	0	0	0	0	0	0	0.10	0
E. compressa	0	0.17	0.10	0.25	0.09	0.50	0.05	0.50
E. compressa f. *glabra*	0	0.17	0.10	0	0.09	0	0.05	0
E. cuspidata	0.50	0.17	0.10	0.25	0.09	0	0.05	0
Trinema enchelys	0	0	0.10	0.25	0.09	0	0.05	0
T. lineare	0	0	0.10	0	0.09	0	0.24	0
Difflugiella oviformis	0.50	0.17	0.10	0	0.09	0	0.05	0
Phryganella acropodia	0	0	0.10	0	0.09	0	0.10	0
Heleopera petricola	0	0	0	0	0.09	0	0.05	0
Centropyxis aerophila	0	0.17	0.10	0	0.09	0	0.10	0
C. sylvatica	0	0	0.10	0	0.09	0	0.05	0
No. of Species	2	6	10		11		13	
No. of individuals/g dry wt	178	204	540	216	1782*	324	3341*	317
% H_2O (wet wt)	62.1	20.1	38.3		58.9		59.1	

* Indicates a significant difference (P = 0.05) from the value recorded at previous sampling time.

Table 5. Fluctuations in numbers of live individuals per species (active plus encysted forms) given in numbers per gram dry weight of litter

Populus tremuloides

	5 mos	8 mos	10 mos	12 mos	18 mos
Arcella catinus	0	34	100	129	175
Euglypha laevis	0	34	100	257	300
E. rotunda	0	34	200	384	844**
E. scutigera	0	0	0	129	100
E. compressa	0	0	0	396	300
E. compressa f. *glabra*	0	0	0	129	100
E. cuspidata	91	34	100	257	374**
Trinema enchelys	0	0	100	129	471**
T. lineare	0	0	50	129	1315**
Difflugiella oviformis	91	34	100	129	300**
Phryganella acropodia	0	0	50	129	100
Heleopera petricola	0	0	0	0	300
Centropyxis aerophila	0	0	0	129	400**
C. aerophila var. *sphagnicola*	0	0	0	0	100
C. sylvatica	0	34	50	129	200
Totals	182	204	850*	2455*	5379*
% Moisture	63.1	16.8	33.4	48.4	58.0

Populus balsamifera

	5 mos	8 mos	10 mos	12 mos	18 mos
Arcella catinus	0	34	108	324	483**
Euglypha laevis	0	0	0	0	157
E. rotunda	0	0	0	0	316
E. scutigera					
E. compressa	0	34	108	324	316
E. compressa f. *glabra*	0	34	54	162	157
E. cuspidata	89	34	108	162	157
Trinema enchelys	0	0	108	162	157
T. lineare	0	0	54	162	796**
Difflugiella oviformis	89	34	54	162	157
Phryganella acropodia	0	0	54	162	324**
Heleopera petricola	0	0	0	162	157
Centropyxis aerophila	0	34	54	162	324**
C. aerophila var. *sphagnicola*					
C. sylvatica	0	0	54	162	157
Totals	178	204	756*	2106*	3658*
% Moisture	62.1	20.1	38.3	58.9	59.1

* Indicates a significant difference (P = 0.05) from the value recorded at previous sampling time

** Indicates significant production (calculated as per Lousier -1974-).

Table 6. Standing crop (numbers and dry weight) of Testacea and bacteria per m^2 of the annual increment of poplar leaf litter.

TIME (Months after burial)	TESTACEA Numbers ($\times 10^6$) Actives	Cysts	Total Biomass* (mg)	BACTERIA Numbers ($\times 10^6$)	Biomass (mg)
0	0	0	0	2.13	7.84×10^{-4}
1	0	0	0	312.2	0.12**
5	0.045	0	0.28**	453.786	166**
8	0.050	0	0.31	-	-
10	0.137	0.072	1.28**	-	-
12	0.396	0.206	3.70**	1.825.375	683
18	1.182	0.103	7.89**	-	-

* Assuming a wet weight of 61.4 mg/10^6 Testacea (Volz, 1951) and a proportion of 90 % water in the testacean protoplasm.

** Indicates significant increases ($P = 0.05$) in biomass from previous sampling time.